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5 **Changes in the sorption-desorption of fungicides over time in an amended sandy clay**
6 **loam soil under laboratory conditions**

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25 **Abstract**

26 *Purpose:* The aim of this work was to study the temporal changes in the sorption-desorption
27 of fungicides in a sandy clay loam soil amended with spent mushroom substrate (SMS) under
28 controlled laboratory conditions and the influence that fungicides properties and soil
29 characteristics have on these processes. Soil amendment with SMS is becoming a widespread
30 management practice since it can effectively solve the problems of uncontrolled SMS
31 accumulation and disposal and improve soil quality. However, when simultaneously applied
32 with pesticides, SMS can significantly modify the environmental behaviour of these
33 compounds.

34 *Materials and methods:* Sorption-desorption isotherms of metalaxyl, penconazole,
35 pyrimethanil and iprovalicarb for an unamended and amended vineyard soil from La Rioja
36 (Spain) were obtained. Composted SMS (C-SMS) and fresh SMS (F-SMS) from cultivation
37 of different mushrooms were used as amendments at 2% and 10% rates. Soil parameters
38 (organic carbon (OC), dissolved organic carbon (DOC), humic acid (HA) and fulvic acid
39 (FA)) and sorption (K_f , n_f , K_d , K_{oc}) and desorption (K_{fd} , n_{fd} , H) parameters of fungicides
40 were determined over 0, 6 and 12 months of soil incubation with SMS under controlled
41 conditions.

42 *Results and discussion:* Addition of amendments to soil increased soil sorption capacity of
43 fungicides. K_d values increased with the hydrophobic character of fungicides (metalaxyl <
44 iprovalicarb < pyrimethanil < penconazole) at both amendment rates. The lower content of
45 DOC and the higher degree of OC humification enhanced sorption of all fungicides by the
46 soil+C-SMS with regard to the soil+F-SMS. In general, sorption of fungicides decreased after
47 6 and 12 months of soil+SMS incubation, although the humification degree of the remaining
48 OC expressed by HA/FA increased. This might indicate that the OC content was more
49 important for fungicide sorption than the changes in its nature with the incubation time. SMS

50 addition favoured desorption of metalaxyl and iprovalicarb, in general, whereas irreversible
51 sorption of penconazole and pyrimethanil increased. However, the opposite trends were
52 observed when the soil+SMS incubation time increased.

53 *Conclusions:* The results show an increase in sorption of all fungicides by amending soil with
54 composted or fresh SMS. However, desorption of fungicides increases or decreases
55 depending on the properties of fungicides and soil+SMS. Changes in both processes with the
56 incubation time are more related to the OC content of the amended soil than to the evolution
57 of its nature. These outcomes are of interest for extending SMS application to soil with
58 minimal or no environmental risk when used simultaneously with fungicides.

59

60 **Keywords** Desorption • Fungicides • Soil • Soil incubation • Sorption • Spent mushroom
61 substrate

62

63

64 **1 Introduction**

65 The application of organic residues to soil is nowadays an increasingly common agricultural
66 practice due to the environmental problems caused by the uncontrolled accumulation and
67 disposal of such residues and to the well-known benefits for soil fertility in terms of organic
68 matter (OM) and nutrient soil enrichment (García-Izquierdo and Lobo 2008). This practice is
69 also a way to sequester carbon and reduce greenhouse gas emissions (Spokas et al. 2009;
70 Zhang et al. 2012). The organic residues potentially applicable to soil come from household
71 activities (sewage sludge or urban solid waste) and agriculture (crop residues, wine or olive
72 oil production or mushroom cultivation). In recent years, the accumulation of spent
73 mushroom substrate (SMS) has significantly risen due to increased mushroom production in
74 China, USA, France, the Netherlands and Spain (FAOSTAT 2009). Some studies have

75 reported an appropriate use of SMS for different purposes (Wuest et al. 1995), but its use as a
76 soil amendment in a raw or composted state has been the most effective due to the high load
77 of OM and low charge of toxic elements (Chiu et al. 2000).

78 In Spain, mushrooms are grown extensively in the La Rioja region, and SMS is often
79 used as a vineyard soil amendment. Due to intensive vine cultivation, the local soils have
80 been seriously degraded and require ameliorating through soil management activities
81 (Miguéns et al. 2007). However, the OM of these residues may interfere with the dynamics of
82 the pesticides (mainly fungicides) used in the vineyards. Fungicides are widely used to control
83 vine fungal diseases, and their use has increased sharply over the past few years in step with
84 greater vine cultivation. Fungicides reach the amended soil either by direct application to the
85 soil or by the subsequent wash-off from treated plants, and interaction with the OM of the
86 residues can increase or decrease its sorption with respect to the unamended soil (Briceño et
87 al. 2007; de Wilde et al. 2009).

88 The effect of natural soil OM on the environmental behaviour of pesticides has been
89 described (Clapp et al. 2001). However, the nature and composition of the OM of organic
90 residues is so variable that it is practically impossible to predict its effect on the
91 environmental behaviour of pesticides. Of special interest is the sorption-desorption of
92 pesticides by soils and how such processes can be affected by the practice of soil amended
93 with organic residues (Reichenberger et al. 2007, Arias-Estévez et al. 2008). This could have
94 important implications for the harmful presence of pesticides in surface and ground water due
95 to sustained leachability or run-off processes (Fenoll et al. 2011; Rodríguez-Cruz et al. 2011).

96 The literature has often investigated the effect of soil application of different organic
97 residues, such as sewage sludge, urban, winery and olive wastes, and pig or cow manure, on
98 the behaviour of herbicides (Delgado-Moreno 2007, Briceño et al. 2008, Wang et al. 2010,
99 López-Piñeiro et al. 2011), insecticides (Sánchez-Camazano et al. 1997, Sánchez et al. 2003)

100 and fungicides (Fernandes et al. 2006, Ghosh and Singh 2009, Filipe et al. 2010). The results
101 from these studies highlight the importance of the kind and nature of the residue, its
102 manipulation and processing, and the rate of its application to the soil. These studies are
103 normally carried out with soils amended under laboratory conditions or over short incubation
104 times, normally of 1-60 days. Only rarely are studies conducted over longer times, when the
105 OM load of soil decays naturally by mineralization at ambient conditions and evolves to a
106 more stable form by humification.

107 Our group has recently initiated a research project designed to clarify some of these
108 unexplored aspects regarding SMS addition to soil as an amendment. Field experiments with plots
109 amended with SMS from *Agaricus bisporus* cultivation have been performed to evaluate the
110 sorption and mobility parameters of penconazole and metalaxyl by soils and subsoils (Marín-
111 Benito et al. 2009a, b) or the dissipation and persistence parameters of tebuconazole in soil
112 (Herrero-Hernández et al. 2011). The results reveal changes in these processes which modify
113 the behaviour of these fungicides at the beginning of the incubation period with a consequent
114 more probable impact onto the contamination of waters.

115 Accordingly, and with a view to establishing a sound practice for the use of SMS as an
116 amendment in soils, the objectives of this work were as follows: 1) evaluate the changes in
117 the sorption-desorption features of four fungicides of different chemical class used in
118 vineyards, using an SMS-amended sandy clay loam soil over 0, 6 and 12 months of
119 incubation under laboratory conditions; 2) study the effect onto sorption-desorption processes
120 of the application rate of SMS, of the kind of treatment, composted and fresh state, and of the
121 properties of the used SMS and fungicides.

122

123 **2 Materials and methods**

124 2.1 Fungicides

125 Selected characteristics of the fungicides studied are included in Table 1 (Tomlin 2000,
126 FOOTPRINT 2011). Fungicides are solid and belong to different chemical classes.
127 Penconazole (triazole) and metalaxyl (acetylalaninate) were supplied by Novartis Crop Protection
128 AG (Basel, Switzerland). They were used unlabeled (purity \geq 98%) and ^{14}C -labeled with
129 specific activity and purity of 1.02 MBq mg^{-1} and $>98\%$ for ^{14}C -penconazole and 1.37 MBq
130 mg^{-1} and >97.2 for ^{14}C -metalaxyl. Pyrimethanil (pyrimidinamine) and iprovalicarb
131 (carbamate) were supplied by Riëdel de Haën (Hannover, Germany) ($> 97.5\%$ purity).

132

133 2.2 Spent mushroom substrates

134 Two SMSs supplied by INTRAVAL (La Rioja, Spain) were used as soil amendments. The
135 composted SMS (C-SMS) is a commercial SMS (Intracompost SPCH-SPS) from *Agaricus*
136 *bisporus* (75%) and *Pleurotus spp.* (25%) production. This C-SMS is obtained by composting
137 the substrate removed from the mushroom houses in piles (2.5 m high) for several weeks
138 under aerobic conditions. The fresh SMS (F-SMS), from *Lentinula edodes* or Shiitake
139 production, was obtained immediately after being removed from the mushroom houses and
140 used without composting. The SMS samples were dried at 45°C, ground and sieved (< 1 mm)
141 on arrival and stored at room temperature for later use. Their characteristics are shown in
142 Table 2. The pH was determined in SMS/water suspensions (1/2 w/v ratio), ash was
143 determined by loss on ignition at 540°C and OM was calculated as 100 - % ash. The organic
144 carbon (OC) content was determined by dichromate oxidation (Walkley-Black method)
145 (Nelson and Sommers, 1996). The dissolved organic carbon (DOC) was determined in a
146 suspension of SMS in Milli-Q ultrapure water (1/100 w/v ratio) after shaking (24 h) at 20°C,
147 centrifugation (20 min at 12,800 g), and filtering through a 0.45 μm filter (Millex HV₄₇
148 Millipore) using a Shimadzu 5050 carbon analyzer (Shimadzu, Columbia, MD, USA). All the
149 determinations were carried out in triplicate. Total N was determined by the Kjeldahl method

150 (Bremner 1996). Metals (Cd, Cr, Cu, Ni, Pb, Zn and Hg) were determined as indicated in
151 Marin-Benito et al. (2009a).

152

153 2.3 Unamended and amended soils

154 The soil sample was taken from the surface horizon (0-30 cm) in a vineyard in the La Rioja
155 region, specifically located in Aldeanueva (Al) (42°14'0"N latitude and 1°53'0"W longitude).
156 It was air-dried and sieved (< 2 mm), and the soil characteristics were determined by standard
157 analytical methods (MAPA, 1986). Aldeanueva soil is a Fluventic Haplocambids (Soil Survey
158 Staff 2006) and its texture was classified as sandy clay loam. The particle size distribution
159 determined using the pipette method was 64.4% sand, 14.2% silt and 21.4% clay, and the
160 inorganic carbon, determined as CaCO₃ with a Bernard calcimeter, was 11.3%. The pH
161 determined in a soil/water suspension (1/1 w/v ratio) was 7.8 and the OC determined as
162 indicated above for SMS was 0.59. DOC was determined in soil/Milli-Q ultrapure water
163 extracts (1/2 w/v ratio) as previously indicated. In addition, alkali soluble and acid insoluble
164 carbon (humic acid, HA) and alkali and acid soluble carbon (fulvic acid, FA) were also
165 obtained in soil extracts following the traditional method of HA and FA extraction from soil
166 OC (Stevenson 1982).

167 Amended soil specimens were prepared by uniformly mixing Al soil with commercial
168 composted SMS (Al+C-SMS) or with Shiitake fresh SMS (Al+F-SMS) at rates of 2% and
169 10%, respectively, on a dry weight basis (equivalent to ~25 and ~125 t SMS ha⁻¹ considering
170 a soil depth of ~10 cm and a soil density of ~1.3 g cm⁻³). The initial moisture content of the
171 soils was adjusted to 40% of their maximum water holding capacity. Unamended and
172 amended soils (~200 g) were then incubated in beakers at 20±2°C in the dark during 0, 6 and
173 12 months. The soil moisture was kept constant during the entire period of the experiment.

174 The characteristics of the soils incubated for different lengths of time were determined as
175 indicated for the unamended soil (Table 3).

176

177 2.4 Sorption experiments

178 The sorption-desorption isotherms of fungicides for air-dried unamended soils and soils
179 amended with SMS after 0, 6 and 12 months of incubation were obtained using the batch
180 equilibrium technique. Duplicate samples of the soils (5 g) were equilibrated with 10 mL of a
181 Milli-Q ultrapure water solution of each fungicide at concentrations of 1, 5, 10, 15, 20 and 25
182 $\mu\text{g mL}^{-1}$ for penconazole, pyrimethanil and metalaxyl and 0.5, 1, 2, 3, 4 and 5 $\mu\text{g mL}^{-1}$ for
183 iprovalicarb. An activity of 100 Bq mL^{-1} was used for ^{14}C -penconazole and ^{14}C -metalaxyl.
184 The suspensions were shaken at $20\pm 2^\circ\text{C}$ for 24 h in a thermostated chamber, with intermittent
185 shaking for 2 h at three-hour intervals. Preliminary experiments revealed that contact for 24 h
186 was long enough for attaining equilibrium. The suspensions were subsequently centrifuged at
187 5045 g for 15-30 min, and the supernatant was filtered through a 0.45 μm filter (Millex HV₄₇
188 Millipore). The equilibrium concentrations of the fungicides in the supernatant were then
189 determined by liquid scintillation counting or high-performance liquid chromatography
190 (HPLC)/mass spectrometry (MS) as indicated in section 2.5. The amount of fungicide sorbed
191 was the difference between that initially present in solution and that remaining after
192 equilibration with the soil.

193 The desorption isotherms of the fungicides were obtained from samples initially
194 treated with 25 $\mu\text{g mL}^{-1}$ of penconazole, pyrimethanil and metalaxyl solution or 5 $\mu\text{g mL}^{-1}$ of
195 iprovalicarb solution. After the samples had been shaken and centrifuged as indicated for the
196 sorption, 5 mL of supernatant was immediately replaced by 5 mL of ultrapure water. The
197 resuspended samples were shaken at $20\pm 2^\circ\text{C}$ for 24 h, after which they were centrifuged and
198 the amount of fungicide desorbed was measured. This desorption procedure was repeated four

199 times for each sample. The amount of fungicide sorbed by the soils at each desorption stage
200 was calculated as the difference between the initial amount sorbed and the amount desorbed.

201

202 2.5 Fungicide analysis

203 The quantitative determination of ^{14}C -penconazole and ^{14}C -metalaxyl after sorption was made
204 on a Beckman LS6500 liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA).

205 The radioactivity of the equilibrium solution was measured in disintegrations per minute
206 (dpm), being determined in 1 mL of supernatant to which 4 mL of scintillation cocktail had
207 been added. The dpm value recorded was related to the dpm obtained for aliquots of the
208 respective standards of the fungicide solutions. These determinations were carried out in
209 duplicate for all the solutions, and the coefficient of variation was always $< 2\%$.

210 Pyrimethanil and iprovalicarb were quantified by HPLC with diode array (DAD) and
211 mass spectrometer (MS) detectors (Waters Association, Milford, MA), using a Waters
212 Symmetry C18 column (75 x 4.6 mm inner diameter, 3.5 μm) at ambient temperature. The
213 mobile phase was 80/20 (v/v) methanol/water (0.1% formic acid) for pyrimethanil, and 60/40
214 acetonitrile/water (0.1% formic acid) for iprovalicarb. The flow rate of the mobile phase was
215 0.3 mL min^{-1} and the sample injection volume was 20 μL . Calibration curves were generated
216 from 0.05-2.0 $\mu\text{g mL}^{-1}$ (pyrimethanil) and 0.125-2.0 $\mu\text{g mL}^{-1}$ (iprovalicarb) concentrations of
217 standards in solutions of sorbent extracts to counteract any possible matrix effect. Retention
218 time was 6.0 and 7.1 min for pyrimethanil and iprovalicarb, respectively, and the limits of
219 detection (LOD) and quantification (LOQ) were, respectively, 0.54 and 1.78 $\mu\text{g L}^{-1}$ for
220 pyrimethanil determination and 0.69 and 2.27 $\mu\text{g L}^{-1}$ for iprovalicarb determination.
221 Quantitative analysis was performed using the peak area of each compound obtained from the
222 total ion chromatogram (TIC) in SIM mode. The positive molecular ion (m/z) was 200.18 and
223 321.30, for pyrimethanil and iprovalicarb, respectively.

224

225 2.6 Data analysis

226 The sorption data of the fungicides were fitted to the linearised form of the Freundlich
227 equation: $\log C_s = \log K_f + n_f \log C_e$, where C_s ($\mu\text{g g}^{-1}$) is the amount of sorbed fungicide;
228 C_e ($\mu\text{g mL}^{-1}$) is the equilibrium concentration of fungicide in solution, and K_f ($\mu\text{g}^{1-n_f} \text{g}^{-1} \text{mL}^{n_f}$)
229 and n_f are the Freundlich sorption and non-linearity coefficients, respectively. K_d (mL g^{-1})
230 distribution coefficients were also determined from the relationship between C_s and C_e , for a
231 C_e of $5 \mu\text{g mL}^{-1}$ for metalaxyl, penconazole and pyrimethanil and $2 \mu\text{g mL}^{-1}$ for iprovalicarb
232 because of the non-linearity of isotherms. K_f values normalised to 100% OC (K_{oc}) were
233 calculated as $K_d 100/\%OC$. Standard deviation (SD) was used to indicate variability in the
234 sorption coefficients among replicates. Sorption constants values were subjected to an analysis
235 of variance, and the least significant difference (LSD), at a confidence level of 95%, was
236 determined to measure the effects of different treatments on the sorption of fungicides by soils.

237

238 **3 Results and discussion**

239 3.1 Sorption of fungicides by non-incubated soil

240 The sorption isotherms of fungicides for AI soil amended with SMS at rates of 2% and 10%
241 immediately after its incorporation in soil (T 0m) and in unamended soil are reported in Fig.
242 1. Isotherms are generally L-type (non-linear) and resemble a C-type, indicating that different
243 mechanisms of sorption may exist between fungicides and soil components and/or residue
244 moieties (Chiou et al. 2000). This kind of isotherm has frequently been found in the sorption
245 of other pesticides that are not extremely hydrophobic, such as triazines, organophosphates,
246 phenylureas, or compounds with chemical groups similar to those of the fungicides
247 considered (Wauchope et al. 2002).

248 The sorption isotherms fit the Freundlich equation, $r \geq 0.98$ ($p < 0.01$), and the K_f and
249 n_f parameters were determined from the linear form of this equation. The n_f values were 0.89
250 (metalaxyl), 0.83 (penconazole), 0.74 (pyrimethanil) and 0.95 (iprovalicarb) in the case of the
251 unamended soil, and a slight increase was observed when soil was amended with both rates of
252 SMS.

253 The K_f sorption constants of the fungicides by the unamended soil varied from 0.24 to
254 5.17, increasing in the following order: metalaxyl < iprovalicarb < pyrimethanil <
255 penconazole, according to the K_{ow} values of the fungicides, except in the case of
256 iprovalicarb. A similar sorption pathway was observed for SMS-amended soils (Table 4); that
257 is, sorption increased as the hydrophobic nature of the fungicide rose. The K_d distribution
258 coefficients were determined to compare the sorption capacity of fungicides by soil because
259 n_f values were different to 1. They varied between 0.20 and 3.95 for the unamended soil
260 (Table 5), and a significant increase was also observed when SMS was added (LSD = 0.012, p
261 < 0.001 for metalaxyl, LSD = 0.74, $p < 0.001$ for penconazole, LSD = 1.19, $p < 0.001$ for
262 pyrimethanil and LSD = 0.33, $p < 0.01$ for iprovalicarb) especially at the higher rate (10%).
263 The K_d constant for the non-incubated soil (time zero, T_{0m}) amended with C-SMS increased
264 by 2.45-6.50 times for metalaxyl, 2.47-6.71 times for penconazole, 2.61-8.82 times for
265 pyrimethanil and 1.14-4.52 times for iprovalicarb. When the soil was amended with F-SMS,
266 the increase of the constant was lower: 1.25-3.50 times for metalaxyl, 1.73-3.85 times for
267 penconazole, 1.72-6.15 times for pyrimethanil and up to 3.23 times for iprovalicarb. This
268 seemed to contrast with the observed higher OC content of the Al+F-SMS mixing (see Table
269 3). As reported by previous unpublished studies (Marin-Benito, 2011), the OC content should
270 favour fungicide sorption, as revealed by the positive and significant correlation ($r \geq 0.94$, $p <$
271 0.001) between K_d coefficients and the OC of unamended vineyard soils with OC < 2%.
272 Thus, the greater sorption capacity of the Al+C-SMS soil could be explained by the higher

273 degree of humification of the OC expressed by the elevated value of the ratio HA/FA and/or
274 by the lower content of DOC (see Table 3) relative to the Al+F-SMS soil. These characteristics
275 of the amended soil are related to the main features of the residues applied, C-SMS and F-
276 SMS (see Table 2).

277 The increase in the sorption of the organic compounds by soils due to the rise in the
278 degree of humification of the OM has often been reported in the literature (Ahmad et al.
279 2001). The effects that DOC has on the sorption of pesticides and hydrophobic compounds by
280 soils are contradictory. Some authors (Barriuso et al. 1992; Andrades et al. 2004) report an
281 increase in the sorption of pesticides if organic soluble compounds from DOC are sorbed by
282 soils and give rise to the formation of new hydrophobic surfaces. A decrease in sorption might
283 occur if pesticides interact with the soluble moieties of OM in the soil-solution interface
284 (Müller et al. 2007, Luo et al. 2009) or when the pesticides compete with the soluble organic
285 molecules for the same sorption sites (Briceño et al. 2008, Cox et al. 2007). These effects
286 could explain our results, which indicated decreased fungicide sorption by the amended soil
287 mixtures with the highest DOC load. Likewise, a significant multiple correlation was found
288 for each fungicide between the K_d and the OC and DOC contents of soil mixtures. This
289 correlation evidenced the contrasting effects of the OC and DOC loads of the amended soil on
290 fungicide sorption, as determined by the batch equilibrium technique. The determination
291 coefficients R^2 of the multiple correlation indicated that more than 90% of the variability of
292 the sorption could be explained by the OC and DOC contents for all the fungicides
293 (penconazole > pyrimethanil > metalaxyl > iprovalicarb) (Table 6).

294

295 3.2 Sorption of fungicides by incubated soil

296 Fig. 1 shows the sorption isotherms of the fungicides for the unamended and amended soil
297 at the rates of 2% and 10% after 6 (T 6m) and 12 (T 12m) months of soil+residue

298 incubation. The Freundlich parameters K_f and n_f (see Table 4) and the K_d coefficients (see
299 Table 5) of the fungicides were determined along with the changes in pH, OC, DOC, HA
300 and FA with soil+SMS incubation time (see Table 3). In the case of the soil Al+C-SMS
301 incubated over 12 months, a significant decrease ($p < 0.01$) in OC up to 18% and in DOC up
302 to 59% was observed, while in the case of the soil Al+F-SMS, the decrease was up to 55% for
303 OC and up to 93% for DOC. For the soil Al+F-SMS, the pH increased in parallel with the
304 sharp decrease observed in the OC load to a value close to the pH of the unamended soil. This
305 trend was expected considering the low pH value of the F-SMS material close to 4.5.
306 However, the composition of the remaining OC changed over time. The HA/FA ratio, which
307 is an indicator of the degree of humification of the OC of the Al+C-SMS and Al+F-SMS soils,
308 increased by 1.1-1.3 times and 1.5-2.6 times, respectively. The significant changes observed in
309 the OC and DOC values appeared to be more significant within the first six months of
310 incubation and then levelled off by 12 months of ageing.

311 The n_f values of the sorption isotherms over time varied in a similar way for both
312 unamended and amended soil, keeping close to values < 1 . The K_d values followed the same
313 variation pathway as those recorded at T 0m. With time, the K_d values did not significantly
314 change for the unamended soil, whereas K_d decreased in the amended soils. The K_d values of
315 penconazole significantly decreased up to 1.55-1.61 times after 6 and 12 months of
316 incubation. The values of metalaxyl decreased up to 1.46 times, and of pyrimethanil up to
317 1.82-2.24 times, although the K_d decrease was not significant for soils treated with the lowest
318 rate of SMS (2%) (Al+C-SMS2 and Al+F-SMS2), which had a lower OC content $< 1.5\%$.
319 The changes in the K_d values for iprovalicarb were quite irregular, decreasing in some cases
320 up to 2.62-2.53 times, and increasing in others (see Table 5).

321 For all fungicides, the greater decrease in K_d corresponded to the soil amended with
322 the fresh residue at a rate of 10% (Al+F-SMS10), which is also the soil-SMS combination

323 with a more consistent decrease in the OC load. This mixture also recorded a very significant
324 increase by up to threefold in the degree of humification of OC (see Table 3). The sorption of
325 the fungicides studied appeared to be linked more closely to the decrease in the OC load of
326 the amended soil than to the changes in the nature or structure of OC. Consistent with these
327 findings, we found a significant linear regression between the K_d and the OC contents of the
328 soil for all the fungicides studied, with correlation coefficient (r) values between 0.91-0.97 (p
329 < 0.05) for soil incubated for six months (T 6m) and 0.88-0.99 ($p < 0.05$) for soil incubated
330 for 12 months (T 12m). However, the latter changes after 12 months of incubation were also
331 consistent with a significant correlation between K_d and the HA/FA ratio of the soil ($r > 0.97$,
332 $p < 0.05$ for metalaxyl; $r > 0.95$, $p < 0.05$ for penconazole, $r > 0.97$, $p < 0.05$ for pyrimethanil
333 and $r > 0.96$, $p < 0.05$ for iprovalicarb).

334 Likewise, other authors have reported a decrease in the sorption of triazines and diuron
335 in soils amended with residues from the olive-mill or wine industries over a short time span
336 (1.5-3 months) (Delgado-Moreno et al. 2007, Fernández-Bayo et al. 2009) even though the
337 OC load remained practically unchanged. Other factors, such as the type of soil and the nature
338 of the DOC added with the organic residue, were also proposed by Cox et al. (2004), and
339 Fernandes et al. (2006) to explain the increase or decrease in the sorption of metalaxyl by
340 soils incubated for two months with different types of amendments.

341 The sorption coefficients were normalised regarding the OC content (K_{oc}) of the soil-
342 SMS mixtures to evaluate the effect on sorption of different characteristics or types of OC.
343 Mean K_{oc} values and their relative coefficients of variation (CV) were obtained to evaluate
344 the K_{oc} variability of fungicides over time (see Table 5). The K_{oc} values recorded a CV in
345 the 24-51% range for the unamended and amended soils at T 0m, revealing the influence the
346 nature of the OC of the residues has on the sorption of fungicides. K_{oc} variability decreased
347 with incubation time and CV varied in the 9.1-18.1% range for the sorption of metalaxyl,

348 penconazole and pyrimethanil (fungicides with Kow values of 1.75-3.72). This highlighted
349 the importance of the OC load after its evolution/stabilization process within the 12-month
350 incubation of soils, rather than the OC nature of the amended soil, which was quite similar
351 after this time. Iprovalicarb (Kow = 3.20) was an exception with a CV of 39.8-53.6%. Even
352 though this fungicide has a slightly hydrophobic character, other soil factors besides OC
353 might influence the sorption of iprovalicarb by the amended soils, as also reported by Sheng
354 et al. (2001) for other carbamates.

355

356 3.3 Desorption of fungicides after 0, 6 and 12 months of incubation

357 The reversibility of fungicides sorbed by amended soils was studied by desorption isotherms
358 from samples initially treated with 25 $\mu\text{g mL}^{-1}$ of penconazole, pyrimethanil and metalaxyl
359 solution or 5 $\mu\text{g mL}^{-1}$ of iprovalicarb solution after 0, 6 and 12 months of soil+residue
360 incubation (see Fig. 1). Kfd and nfd desorption parameters (Table 7) were obtained from the
361 Freundlich equation ($r \geq 0.87$, $p < 0.01$) and hysteresis coefficients (H) were determined ($H =$
362 n_f/n_{fd}) (Table 8). Desorption isotherms showed hysteresis, as the desorption data did not
363 match those of the sorption isotherm, and the hysteresis coefficients obtained were constantly
364 > 1 for both unamended and amended soils, indicating that the desorption process was not
365 fully reversible.

366 For the unamended soil, the values of H were in the 1.10-10.1 range, with peak values
367 observed for metalaxyl and iprovalicarb. The values of H for the desorption of penconazole
368 (2.66-8.18) and pyrimethanil (1.51-4.40) were higher for the amended soil than for the
369 unamended one, especially in the case of the soil Al+C-SMS at the higher SMS rate.
370 However, the hysteresis of the desorption isotherms of metalaxyl (1.44-2.93) and
371 iprovalicarb (1.32-4.39) from the amended soils was similar or decreased with respect to
372 the unamended soil. The irreversibility of sorption of these fungicides decreased with the

373 increase in the SMS rate, indicating that the organic residue facilitated the compound's
374 desorption. After six months of incubation, a significant decrease in H was observed for
375 penconazole, with an increase for pyrimethanil regarding the values at T 0m. After 12
376 months, an increase in H for metalaxyl and iprovalicarb and a decrease for pyrimethanil were
377 recorded, whereas no significant changes were observed for penconazole as regards the values
378 at T 6 m.

379 The amounts of each fungicide desorbed from unamended and amended soils after four
380 consecutive desorption cycles and over the three different times of incubation are shown in
381 Fig. 2. They are reported as percentages of fungicides with respect to the initial amounts
382 sorbed, varying in the 41.8-75.8% range for metalaxyl, 34.9-35.9% for penconazole, 48.5-
383 69.1% for pyrimethanil and 17.0-24.5% for iprovalicarb from the unamended soil, and in the
384 51.4-76.0% range for metalaxyl, 6.99-29.1% for penconazole, 15.8-57.6% for pyrimethanil
385 and 30.5-79.4% for iprovalicarb from the amended soils.

386 The data reveal that the increase in OC due to the application of the organic residues
387 led to a decrease in the desorption of penconazole and pyrimethanil from the amended soil
388 with both rates of residues, although the effect was greater for the soil amended with C-SMS
389 at a rate of 10%. The decrease in soil OC content after incubation caused a constant increase
390 in penconazole desorption from the amended soil, and a variable effect on the pyrimethanil
391 desorption was reported. The desorption of metalaxyl from the amended soil increased or was
392 similar to that from the unamended soil, increasing after six months' incubation with F-SMS
393 and decreasing after 12 months' incubation in all amended soils. The desorption of
394 iprovalicarb increased with the amendment applied to all the soils, as well as after six months'
395 incubation, and then started to decrease. This contrasting behaviour of the fungicides is
396 probably linked to their different interaction mechanisms with the residues or with the soil
397 components. The less hydrophobic fungicides pyrimethanil, metalaxyl and iprovalicarb might

398 interact with specific functional groups of the residue or with the organic and inorganic
399 moieties of the soil. Penconazole could be sorbed mainly by OM through a hydrophobic
400 mechanism. These interaction mechanisms have been reported by Marin-Benito et al. (2012)
401 in a FTIR study of the fungicides sorbed by SMS without soil.

402

403 **4 Conclusions**

404 The results reveal how SMS increases the sorption capacity of fungicides of different
405 chemical classes and properties by soil amended with it at a rate of $\geq 2\%$. The lower DOC
406 load and the greater degree of humification of the OC of the SMS sustained the higher
407 sorption of all the fungicides by the soil amended with the composted SMS. The incubation of
408 soil+SMS for 6-12 months decreased fungicide sorption, especially for the soil amended with
409 fresh SMS at a rate of 10%. The decrease in the load of OC with incubation time was more
410 important for explaining fungicide sorption behaviour than the changes in OC nature and
411 structure. SMS addition enhanced the desorption of metalaxyl and iprovalicarb, but decreased
412 that of penconazole and pyrimethanil. However, the opposite effects were observed when
413 incubation time was increased. On the whole, the percentages of desorption were below 100%
414 of the initial amount sorbed for all the fungicides. These results might sustain the practice of
415 SMS addition especially in the vineyard to favour fungicide retention by soils, even after
416 extended incubation times. This will optimise the field use of SMS reducing the leaching
417 probability of fungicides with a different hydrophobicity to groundwater.

418

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425

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547 **Figure legends**

548

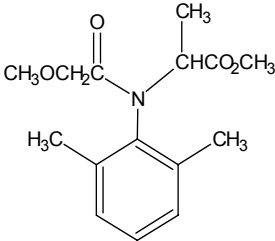
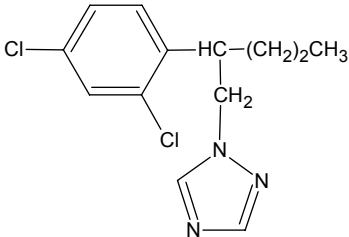
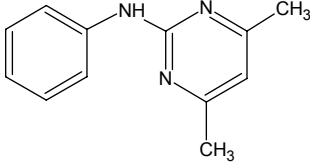
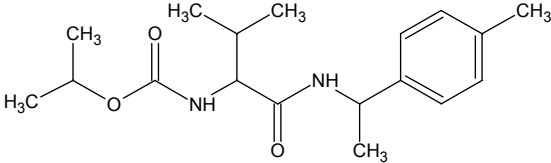
549 **Fig. 1** Sorption-desorption isotherms of fungicides in unamended soil (Al), soil amended
550 with composted spent mushroom substrate at rates of 2% (*Al+C-SMS2*) and 10% (*Al+C-*
551 *SMS10*), and soil amended with fresh spent mushroom substrate at rates of 2% (*Al+F-SMS2*)
552 and 10% (*Al+F-SMS10*). Error bars represent standard deviation of the mean value

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554 **Fig. 2** Desorbed amounts of fungicide from unamended soil (Al), soil amended with
555 composted spent mushroom substrate at rates of 2% (*Al+C-SMS2*) and 10% (*Al+C-SMS10*),
556 and soil amended with fresh spent mushroom substrate at rates of 2% (*Al+F-SMS2*) and 10%
557 (*Al+F-SMS10*) initially treated with 25 mg L⁻¹ of penconazole, pyrimethanil and metalaxyl
558 solution or 5 mg L⁻¹ of iprovalicarb solution after four successive desorption stages and at 0, 6
559 and 12 months of soil+SMS incubation. Error bars represent standard deviation of the mean
560 value

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Table 1. Chemical structure and characteristics of fungicides

Common name Chemical name	Chemical structure	WS (mg L ⁻¹) ^a	log Kow ^a	Koc (mL g ⁻¹) ^a	Half life (days ⁻¹) ^a
Metalaxyl		8400	1.75	28-284	42-46
Methyl <i>N</i> -methoxyacetyl- <i>N</i> -2,6-xylol-DL-alaninate					
Penconazole		73	3.72	786-4120	22-207
1-(2,4-dichloro-β-propylphenetyl)-1 <i>H</i> -1,2,4-triazole					
Pyrimethanil		121	2.84	75-500	23-71.8
<i>N</i> -(4,6-dimethylpyrimidin-2-yl) aniline					
Iprovalicarb		11	3.2	106	4.7-30
Isopropyl 2-methyl-1-[(1- <i>p</i> -tolylethyl)carbamoyl]-(<i>S</i>)-propylcarbamate					

568 WS Solubility in water at 20°C, Kow Octanol/water partition coefficient at pH 7 and 20°C, Koc
569 Sorption coefficient normalized to organic carbon content. ^aFrom FOOTPRINT (2011) and
570 Tomlin (2000)

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Table 2. Characteristics of spent mushroom substrates given on a dry weigh basis

Characteristics	C-SMS	F-SMS
pH (1:2 ratio)	7.5	4.5
Ash %	48.3	32.5
Moisture %	32.5	55.3
OM (100-ash%)	51.7	67.5
OC %	26.7	31.2
N total (%)	2.24	1.75
C/N	12.5	17.9
DOC %	1.22	10.8
HA (%)	3.21	4.52
FA (%)	1.32	9.21
HA/FA	2.43	0.49
Cd (mg/kg)	0.32	0.14
Cr (mg/kg)	89.4	3.72
Cu (mg/kg)	38.1	5.76
Ni (mg/kg)	28.4	2.42
Pb (mg/kg)	29.6	2.78
Zn (mg/kg)	180	292
Hg (mg/kg)	0.03	<0.01

OM organic matter, OC organic carbon, DOC water soluble organic carbon, HA alkali soluble organic carbon, FA acid and alkali soluble organic carbon, C-SMS composted spent mushroom substrate, F-SMS fresh spent mushroom substrate

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Table 3. Characteristics of unamended and amended soils after 0, 6 and 12 months of incubation

Time of treatment/Soil	pH	OC (%)	DOC (mg g ⁻¹)	HA (mg g ⁻¹)	FA (mg g ⁻¹)	HA/FA
0 months						
Al	7.87	0.59	0.046	0.432	0.563	0.767
Al+C-SMS2	7.73	1.28	0.193	0.670	0.754	0.888
Al+C-SMS10	7.44	3.81	0.817	2.079	1.872	1.110
Al+F-SMS2	7.26	1.43	1.120	0.764	2.170	0.352
Al+F-SMS10	6.04	3.96	10.47	2.662	9.562	0.278
6 months						
Al	7.89	0.53	0.045	-	-	-
Al+C-SMS2	7.70	1.10	0.204	-	-	-
Al+C-SMS10	7.41	3.10	0.425	-	-	-
Al+F-SMS2	7.64	0.90	0.119	-	-	-
Al+F-SMS10	7.70	1.95	1.007	-	-	-
12 months						
Al	7.84	0.58	0.036	0.357	0.621	0.575
Al+C-SMS2	7.65	1.18	0.079	0.717	0.758	0.946
Al+C-SMS10	7.47	3.12	0.364	2.439	1.631	1.495
Al+F-SMS2	7.88	0.95	0.158	0.526	0.977	0.538
Al+F-SMS10	7.90	1.78	0.706	2.069	2.841	0.728

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Al unamended soil, Al+C-SMS2 soil amended with composted spent mushroom substrate at rate of 2%, Al+C-SMS10 soil amended with composted spent mushroom substrate at rate of 10%, Al+F-SMS2 soil amended with fresh spent mushroom substrate at rate of 2%, Al+F-SMS10 soil amended with fresh spent mushroom substrate at rate of 10%

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Table 4. Ranges of Freundlich constants for the sorption of fungicides by unamended Al soil and Al soil amended with composted (Al+C-SMS10 and Al+C-SMS2) and fresh (Al+F-SMS10 and Al+F-SMS2) spent mushroom substrate after 0, 6 and 12 months of incubation.

Fungicide	0 months		6 months		12 months	
	Kf (SD)	nf (SD)	Kf (SD)	nf (SD)	Kf (SD)	nf (SD)
Metalaxyl	0.23-1.56 (0.01-0.07)	0.81-1.06 (0.01-0.04)	0.27-1.52 (0.01-0.02)	0.86-0.94 (0.00-0.03)	0.23-1.21 (0.00-0.02)	0.87-0.95 (0.00-0.04)
Penconazole	5.17-31.8 (0.00-0.39)	0.83-0.96 (0.00-0.02)	4.88-26.6 (0.02-0.64)	0.83-0.90 (0.00-0.02)	4.67-23.5 (0.00-0.18)	0.82-0.92 (0.00-0.01)
Pyrimethanil	1.70-12.6 (0.10-0.58)	0.74-0.92 (0.04-0.09)	1.71-10.4 (0.03-0.33)	0.85-0.94 (0.00-0.07)	1.81-10.2 (0.00-0.22)	0.80-0.89 (0.00-0.06)
Iprovalicarb	0.44-3.41 (0.01-0.15)	0.72-1.05 (0.01-0.19)	0.48-3.24 (0.00-0.09)	0.86-1.00 (0.01-0.18)	0.19-2.45 (0.02-0.03)	0.83-1.07 (0.01-0.08)

636 Al+C-SMS2 soil amended with composted spent mushroom substrate at rate of 2%, Al+C-
 637 SMS10 soil amended with composted spent mushroom substrate at rate of 10%, Al+F-SMS2 soil
 638 amended with fresh spent mushroom substrate at rate of 2%, Al+F-SMS10 soil amended with
 639 fresh spent mushroom substrate at rate of 10%, Ranges of standard deviations (SD) of Kf and nf
 640 Freundlich sorption constants are indicated in parenthesis.
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Table 5. Distribution coefficients (Kd) for the sorption of fungicides by unamended soil and soil amended with composted and fresh spent mushroom substrate after 0, 6 and 12 months of incubation and organic carbon normalised coefficients (Koc)

Fungicide Soil	0 months		6 months		12 months	
	Kd±SD	Koc	Kd±SD	Koc	Kd±SD	Koc
Metalaxyl						
Al	0.20±0.01a	37.0	0.23±0.00 ^a	43.4	0.22±0.00	37.9
Al+C-SMS2	0.49±0.00c	38.3	0.42±0.01 ^a	38.2	0.43±0.01 ^a	36.4
Al+C-SMS10	1.30±0.01e	34.1	1.22±0.00 ^a	39.4	1.02±0.00 ^a	32.7
Al+F-SMS2	0.25±0.00b	17.5	0.26±0.01	28.9	0.26±0.01	27.4
Al+F-SMS10	0.70±0.00d	17.7	0.48±0.01 ^a	24.6	0.48±0.01 ^a	27.0
Mean		28.9		34.9		32.3
CV (%)		36.2		22.4		15.6
Penconazole						
Al	3.95±0.10a	731	3.73±0.07	703	3.48±0.04 ^a	600
Al+C-SMS2	9.77±0.07b	763	6.88±0.06 ^a	625	7.80±0.13 ^a	661
Al+C-SMS10	26.5±0.45e	695	21.6±0.71 ^a	696	18.7±0.04 ^a	599
Al+F-SMS2	6.83±0.21c	477	5.20±0.27 ^a	577	5.10±0.09 ^a	536
Al+F-SMS10	15.2±0.38d	383	9.80±0.08 ^a	502	9.47±0.08 ^a	532
Mean		610		621		585
CV (%)		27.7		13.6		9.1
Pyrimethanil						
Al	1.12±0.10a	207	1.39±0.07	262	1.32±0.01	227
Al+C-SMS2	2.92±0.26b	228	2.89±0.16	262	2.99±0.09	253
Al+C-SMS10	9.88±0.39d	259	8.84±0.25 ^a	285	8.02±0.17 ^a	257
Al+F-SMS2	1.93±0.10ab	135	1.83±0.03	203	1.73±0.04	182
Al+F-SMS10	6.89±0.91c	174	3.78±0.08 ^a	193	3.07±0.04 ^a	172
Mean		200		241		218
CV (%)		24.0		16.7		18.1
Iprovalicarb						
Al	0.69±0.01ab	128	0.51±0.01	96.2	0.70±0.01	120
Al+C-SMS2	0.79±0.01b	61.7	0.44±0.00 ^a	40.0	1.26±0.04 ^a	106
Al+C-SMS10	3.12±0.07d	81.9	3.05±0.01	98.4	2.34±0.01 ^a	75.0
Al+F-SMS2	0.43±0.06a	30.1	0.64±0.01 ^a	71.1	0.17±0.06 ^a	17.9
Al+F-SMS10	2.23±0.27c	56.3	0.85±0.01 ^a	43.6	1.05±0.04 ^a	59.0
Mean		71.6		69.9		75.9
CV (%)		51.0		39.8		53.6

648 Al unamended soil, Al+C-SMS2 soil amended with composted spent mushroom substrate
649 at rate of 2%, Al+C-SMS10 soil amended with composted spent mushroom substrate
650 at rate of 10%, Al+F-SMS2 soil amended with fresh spent mushroom substrate at rate of 2%,
651 Al+F-SMS10 soil amended with fresh spent mushroom substrate at rate of 10%, Kd
652 distribution coefficients are indicated as mean values ± standard deviation
653 a,b,c,d,e, Kd values at 0 months followed with the same letter are not significantly
654 different (p<0.05), ^a Kd values in the same raw at 6 months and 12 months are
655 significantly different of Kd values at 0 months (p<0.05)
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Table 6. Multiple regression equations between distribution coefficients of fungicides and organic carbon and dissolved organic carbon.

Fungicide	Regression equation	Significance level		R ²
		OC	DOC	
Metalaxyl	Kd = -0.042+0.365 OC – 0.068 DOC	0.020	0.066	96.0
Penconazole	Kd = -0.391+7.322 OC – 1.296 DOC	0.007	0.027	98.6
Pyrimethanil	Kd = -0.897+2.877 OC – 0.353 DOC	0.011	0.082	98.1
Iprovalicarb	Kd = -0.204+0.866 OC – 0.099 DOC	0.049	0.293	91.0

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Kd distribution coefficients of fungicides, OC organic carbon, DOC dissolved organic carbon

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Table 7. Ranges of Freundlich constants for the desorption of fungicides from unamended Al soil and Al soil amended with composted (Al+C-SMS2 and Al+C-SMS10) and fresh (Al+F-SMS2 and Al+F-SMS10) spent mushroom substrate after 0, 6 and 12 months of incubation.

Fungicide	0 months		6 months		12 months	
	Kfd (SD)	nfd (SD)	Kfd (SD)	nfd (SD)	Kfd (SD)	nfd (SD)
Metalaxyl	1.34-3.70 (0.01-0.24)	0.36-0.60 (0.01-0.04)	0.71-3.18 (0.03-0.39)	0.43-0.65 (0.01-0.06)	1.26-3.97 (0.03-0.52)	0.21-0.61 (0.00-0.06)
Penconazole	14.4-44.7 (0.03-2.63)	0.11-0.36 (0.01-0.05)	13.2-42.7 (0.47-1.68)	0.13-0.39 (0.01-0.02)	13.8-40.8 (0.19-0.53)	0.14-0.35 (0.01-0.02)
Pyrimethanil	2.31-31.7 (0.15-1.52)	0.19-0.67 (0.00-0.09)	5.48-27.4 (1.09-2.98)	0.26-0.47 (0.03-0.10)	3.56-22.3 (0.18-0.86)	0.36-0.57 (0.01-0.03)
Iprovalicarb	1.10-3.59 (0.03-0.52)	0.09-0.66 (0.00-0.08)	0.51-3.56 (0.00-0.43)	0.12-0.88 (0.00-0.08)	0.39-3.34 (0.03-0.37)	0.08-1.09 (0.00-0.03)

693 Al+C-SMS2 soil amended with composted spent mushroom substrate at rate of 2%, Al+C-
 694 SMS10 soil amended with composted spent mushroom substrate at rate of 10%, Al+F-SMS2 soil
 695 amended with fresh spent mushroom substrate at rate of 2%, Al+F-SMS10 soil amended with
 696 fresh spent mushroom substrate at rate of 10%, Ranges of standard deviations (SD) of Kfd and
 697 nfd Freundlich desorption constants are indicated in parenthesis
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Table 8. Hysteresis coefficients for the desorption of fungicides by unamended and amended soil after 0, 6 and 12 months of incubation.

Fungicide Soil	0 months H±SD	6 months H±SD	12 months H±SD
Metalaxyl			
Al	2.45±0.22	1.56±0.07	4.59±1.27
Al+C-SMS2	2.04±0.19	2.07±0.36	2.47±0.09
Al+C-SMS10	1.49±0.02	1.47±0.02	1.87±0.19
Al+F-SMS2	2.93±0.30	1.78±0.10	3.05±0.04
Al+F-SMS10	1.44±0.07	1.36±0.09	1.42±0.19
Penconazole			
Al	2.28±0.26	2.15±0.08	2.35±0.07
Al+C-SMS2	4.99±1.47	3.05±0.08	3.33±0.07
Al+C-SMS10	8.18±0.62	6.75±0.48	6.31±0.46
Al+F-SMS2	2.66±0.16	2.42±0.20	2.51±0.04
Al+F-SMS10	3.39±0.58	2.90±0.30	2.82±0.15
Pyrimethanil			
Al	1.10±0.03	1.94±0.41	1.46±0.04
Al+C-SMS2	1.62±0.19	2.25±0.53	1.63±0.19
Al+C-SMS10	4.40±0.02	3.49±0.67	2.37±0.06
Al+F-SMS2	1.51±0.15	1.90±0.34	1.79±0.07
Al+F-SMS10	1.58±0.09	2.32±0.21	1.57±0.07
Iprovalicarb			
Al	10.1±1.08	7.30±1.69	13.2±0.67
Al+C-SMS2	2.24±0.02	1.38±0.38	7.02±0.22
Al+C-SMS10	1.32±0.06	1.41±0.16	0.86±0.03
Al+F-SMS2	4.39±1.05	1.12±0.25	1.95±0.46
Al+F-SMS10	1.71±0.36	2.14±0.07	3.77±0.47

Al unamended soil, Al+C-SMS2 soil amended with composted spent mushroom substrate at rate of 2%, Al+C-SMS10 soil amended with composted spent mushroom substrate at rate of 10%, Al+F-SMS2 soil amended with fresh spent mushroom substrate at rate of 2%, Al+F-SMS10 soil amended with fresh spent mushroom substrate at rate of 10%, H hysteresis coefficients are indicated as mean values ± standard deviation.

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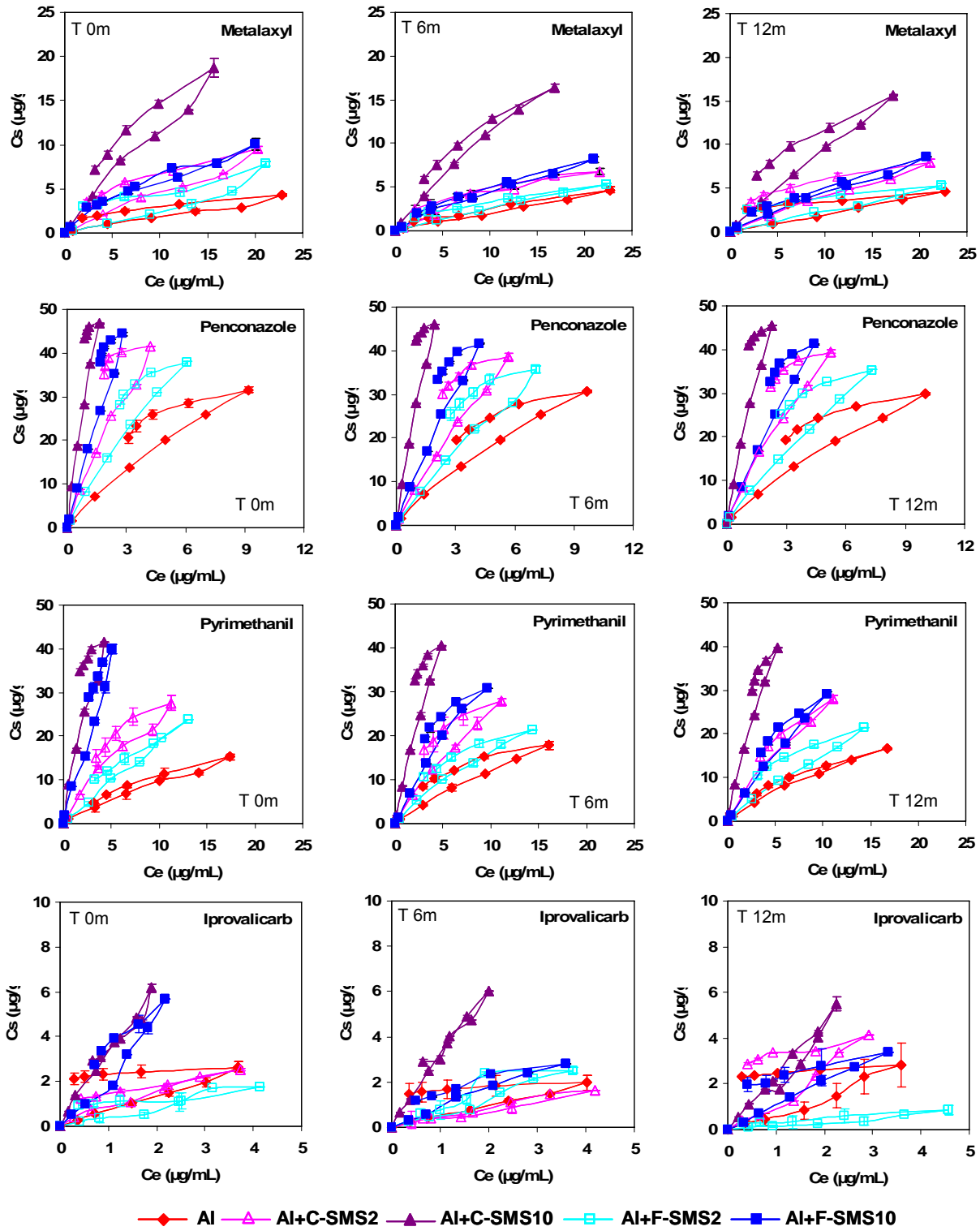


Fig 1

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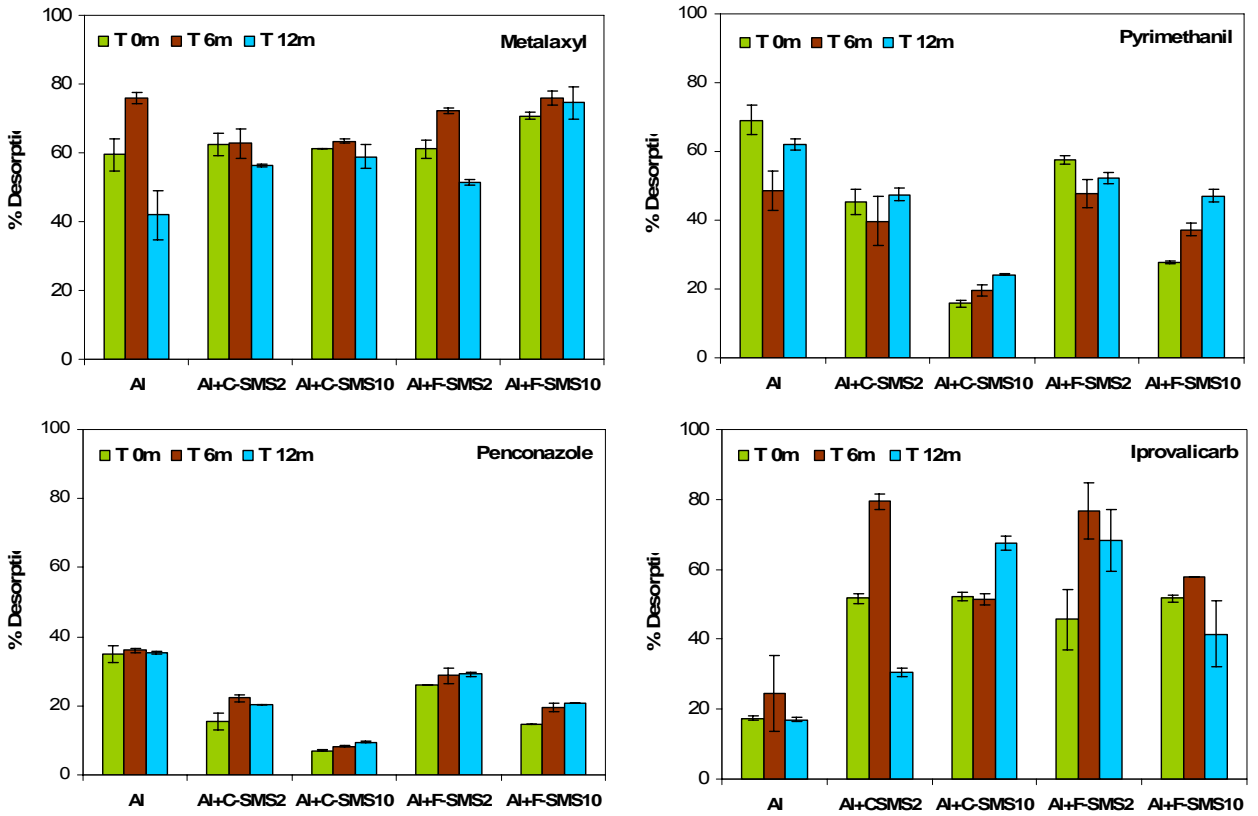


Fig 2